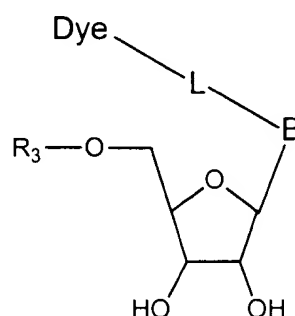


WHAT IS CLAIMED IS:

1. A compound having the formula:

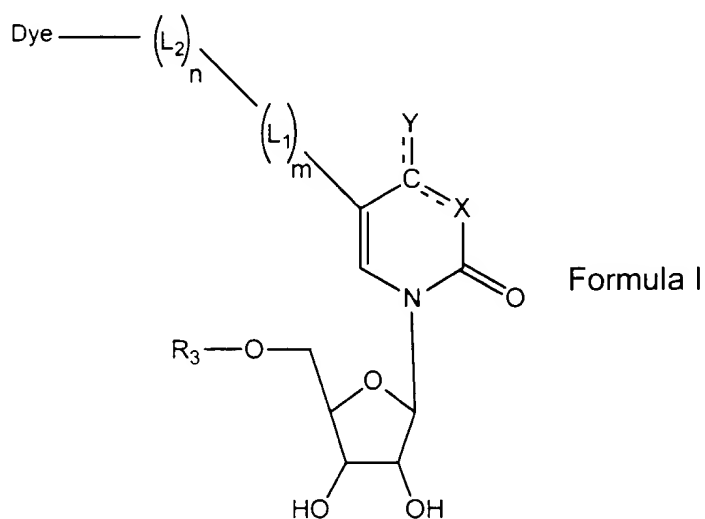


wherein B is a nucleobase; L is a linker; R<sub>3</sub> is triphosphate, α-thiotriphosphate, or a salt thereof, and Dye is a reporter group

2. The compound according to claim 1, wherein the linker is an propargyl-ethyl-oxide-amino linker.
3. The compound according to claim 1, wherein the linker is a propargyl linker.
4. The compound according to claim 1, wherein the linker is a benzylamine linker.
5. The compound according to claim 1, wherein the linker is a phosphate linker.
6. The compound according to claim 1, wherein the linker is a rigid linker.
7. The compound according to claim 1, wherein the linker is a tuned linker.
8. The compound according to claim 1, wherein the linker is a heterocycle linker.

9. The compound according to claim 1, wherein the dye is a fluorescein-type dye, a rhodamine type dye, an energy transfer dye pair, or a cyanine-type dye.
10. The compound according to claim 9, wherein Dye is selected from a rhodamine dye and a fluorescein dye.
11. The compound according to claim 10, wherein the rhodamine dye is a 4,7-dichlorophenyl-rhodamine dye.
12. The compound according to claim 11, wherein the 4,7-dichlorophenyl-rhodamine dye is selected from dTAMRA, dROX, dR6G, and dR110.
13. The compound according to claim 1, wherein the nucleobase is selected from cytosine, adenine, uracil, guanine, 7-deazaadenine, and 7-deazaguanine.

14. A compound of the formula I:



- wherein X is N, NH, or C;
- wherein Y is O or NH<sub>2</sub>;
- wherein R<sub>3</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein L<sub>1</sub> is a linker;
- wherein L<sub>2</sub> is a benzylamine linker or a phosphate linker;
- wherein n = 0-4, m = 0-4, and m + n is at least 1; and;
- wherein the dye is any reporter group.

15. The compound according to claim 14, wherein X is N and Y is NH<sub>2</sub>.

16. The compound according to claim 14, wherein X is C and Y is O.

17. The compound according to claim 14 wherein L<sub>1</sub> is a propargyl-ethyl-oxide-amino linker, a propargylamino linker, a propargyl-propyl-oxide-amino linker, a benzylamine linker, a phosphate linker, a rigid linker, or a multimer thereof.

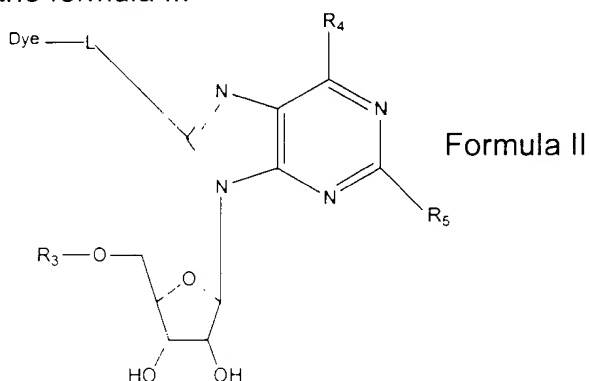
18. The compound according to claim 14, wherein  $m = 1$  and  $L_2$  is a benzylamine linker.

19. The compound according to claim 18, wherein  $L_1$  is a propargyl-ethyl-oxide-amino linker or a propargylamino linker.

20. The compound according to claim 14, wherein  $m = 1$  and  $L_2$  is a phosphate linker.

21. The compound according to claim 20, wherein  $L_1$  is a propargyl-ethyl-oxide-amino linker or a propargylamino linker.

22. A compound of the formula II:



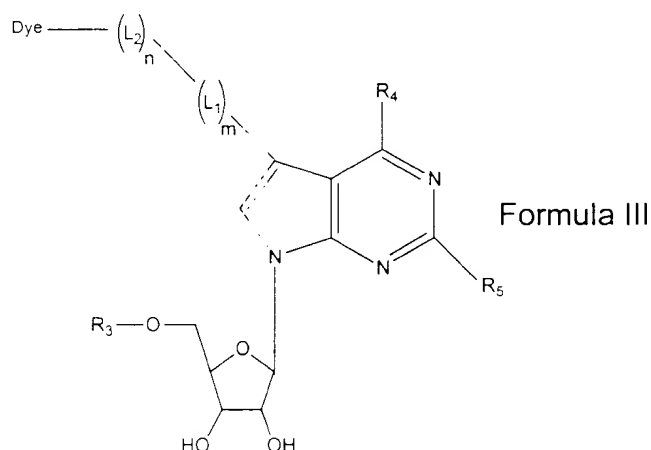
- wherein L is a linker;
- wherein  $R_4$  is either  $NH_2$ , OH, or O, and B is either  $NH_2$ , OH, or H;
- wherein  $R_3$  is either triphosphate,  $\alpha$ -thiotriphosphate, or a salt thereof; and
- wherein the dye is any reporter group.

23. The compound according to claim 22, wherein the linker is a propargyl-ethyl-oxide-amino linker, a propargylamino linker, a propargyl-propyl-oxide-amino linker, a benzylamine linker, a phosphate linker, a rigid linker, or a multimer thereof.

24. The compound according claim 22, wherein the linker is an propargyl-ethyl-oxide-amino linker.

25. The compound according to claim 22, wherein the linker is a propargylamino linker.

26. A compound of the formula III:



- wherein  $L_1$  is a linker;
- wherein  $L_2$  is a benzylamine linker or a phosphate linker;
- wherein  $n = 0-4$ ,  $m = 0-4$ , and  $m + n$  is at least 1;
- wherein  $R_4$  is either  $NH_2$ ,  $OH$ , or  $O$ , and  $R_5$  is either  $NH_2$ ,  $OH$ , or  $H$ ;
- wherein  $R_3$  is either triphosphate,  $\alpha$ -thiotriphosphate, or a salt thereof; and
- wherein the dye is any reporter group.

27. The compound according to claim 26, wherein  $L_1$  is a propargyl-ethyl-oxide-amino linker, a propargylamino linker, a propargyl-propyl-oxide-amino linker, a benzylamine linker, a phosphate linker, a rigid linker, or a multimer thereof.

28. The compound according to claim 26, wherein  $n = 1$  and  $L_2$  is a benzylamine linker.

29. The compound according to claim 28, wherein  $L_1$  is a propargyl-ethyl-oxide-amino linker or a propargylamino linker.

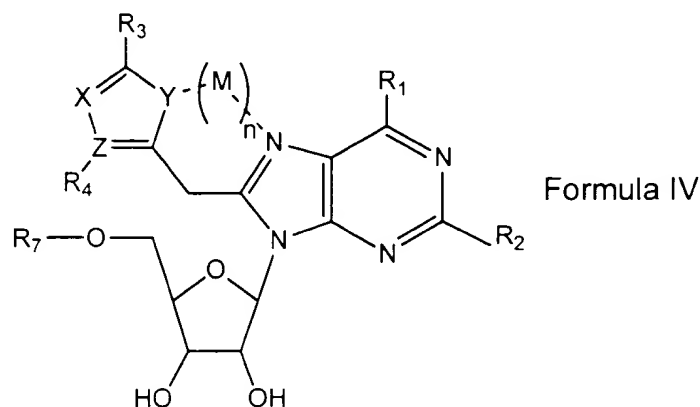
30. The compound according to claim 26, wherein  $n = 1$  and  $L_2$  is a phosphate linker.

31. The compound according to claim 30, wherein  $L_1$  is a propargyl-ethyl-oxide-amino linker or a propargylamino linker.

32. The compound according to claim 28, wherein  $R_4$  is  $NH_2$  and  $R_5$  is H.

33. The compound according to claim 28, wherein  $R_4$  is O and  $R_5$  is  $NH_2$ .

34. A compound of the formula IV:

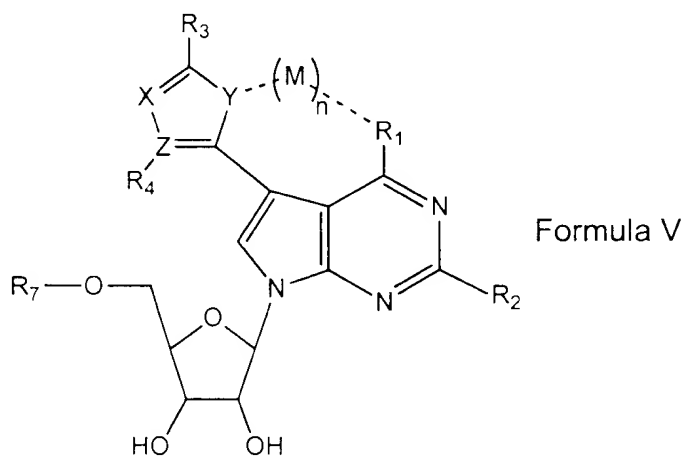


- wherein  $R_1$ ,  $R_2$ , and  $R_4$  are independently H, O, OR, S, SR,  $NR_2$  or  $CR_2$ ;
- wherein  $R_3$  is SR,  $NR_2$ , OR, or  $CR_2$  and comprises a reporter group;
- wherein R is hydrogen, alkyl, aryl, or an amino acid;
- wherein  $R_7$  is either triphosphate,  $\alpha$ -thiotriphosphate, or a salt thereof;
- wherein X, Y, and Z are independently carbon, nitrogen, oxygen, sulfur, phosphorus, or selenium;
- wherein n is 0 or 1; and
- wherein M is  $H_2O$  or any metal.

35. The compound according to claim 34, wherein R is hydrogen, C1-C6 alkyl, C2-C6 alkenyl, C2-C6 alkynyl, benzyl substituted at from 0 to 3 positions in a chemically reasonable manner with F, Cl, Br, I, C1-C18 alkyl, Silyl, OH, OR', SH, SR', SOR',  $SO_2R'$ ,  $SO_3$ , or  $NR'_2$ , or an amino acid and further wherein R' is H, OH, or alkyl.

36. The compound according to claim 34, wherein  $n = 0$ .

37. The compound according to claim 34, wherein  $n = 1$  and further wherein M is a Group IA metal or a Group IIA metal
38. The compound according to claim 37, wherein M is  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ , or  $\text{Ca}^{2+}$ .
39. The compound according to claim 34, wherein  $\text{R}_1$  is  $\text{NH}_2$  and  $\text{R}_2$  is H.
40. The compound according to claim 34, wherein  $\text{R}_1$  is O and  $\text{R}_2$  is  $\text{NH}_2$ .
41. The compound according to claim 35, wherein  $\text{R}'$  is H, OH, C1-C6 alkyl, or C2-C6 alkynyl.
42. A compound of the formula V:



- wherein  $\text{R}_1$ ,  $\text{R}_2$ , and  $\text{R}_4$  are independently H, O, OR, S, SR,  $\text{NR}_2$  or  $\text{CR}_2$ ;
- wherein  $\text{R}_3$  is SR,  $\text{NR}_2$ , OR, or  $\text{CR}_2$  and comprises a reporter group;
- wherein R is hydrogen, alkyl, aryl, or an amino acid;



- wherein  $R_7$  is either triphosphate,  $\alpha$ -thiotriphosphate, or a salt thereof;
- wherein X, Y, and Z are independently carbon, nitrogen, oxygen, sulfur, phosphorus, or selenium;
- wherein n is 0 or 1; and
- wherein M is  $H_2O$  or any metal.

43. The compound according to claim 42, wherein R is hydrogen, C1-C6 alkyl, C2-C6 alkynyl, C2-C6 alkenyl, benzyl substituted at from 0 to 3 positions in a chemically reasonable manner with F, Cl, Br, I, C1-C18 alkyl, Silyl, OH, OR', SH, SR', SOR',  $SO_2R'$ ,  $SO_3$ , or  $NR'_2$ , or an amino acid and further wherein R' is H, OH, or alkyl.

44. The compound according to claim 42, wherein  $n = 0$ .

45. The compound according to claim 42, wherein  $n = 1$  and further wherein M is a Group IA metal or a Group IIA metal

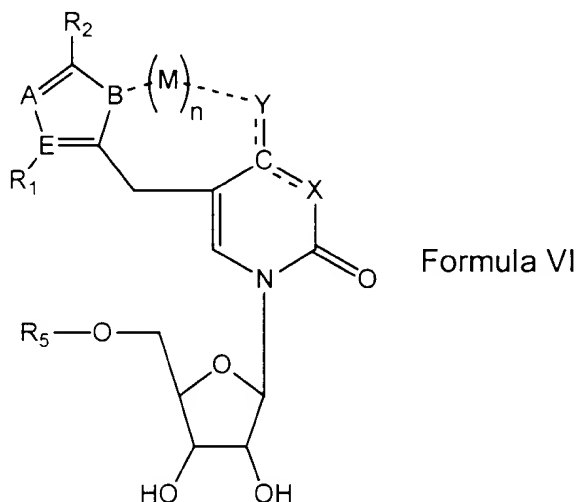
46. The compound according to claim 45, wherein M is  $Li^+$ ,  $Na^+$ ,  $K^+$ ,  $Mg^{2+}$ , or  $Ca^{2+}$ .

47. The compound according to claim 42, wherein  $R_1$  is  $NH_2$  and  $R_2$  is H.

48. The compound according to claim 42, wherein  $R_1$  is O and  $R_2$  is  $NH_2$ .

49. The compound according to claim 43, wherein R' is H, OH, C1-C6 alkyl, or C2-C6 alkynyl.

50. A compound of the formula VI:

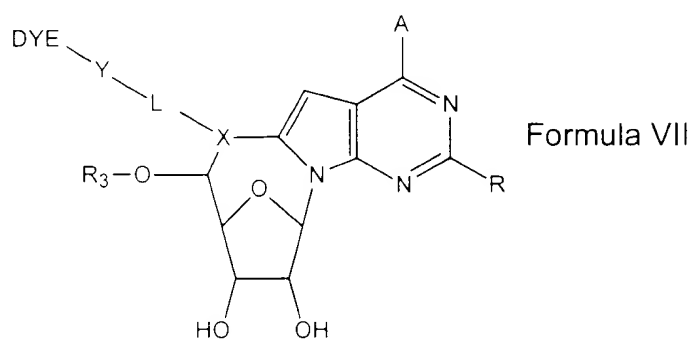


- wherein R<sub>1</sub> is H, O, OR, S, SR, NR<sub>2</sub>, or CR<sub>2</sub>,
- wherein R<sub>2</sub> is SR, NR<sub>2</sub>, OR, or CR<sub>2</sub> and comprises a reporter group;
- wherein R is hydrogen, alkyl, alkynyl, aryl, or an amino acid;
- wherein R<sub>5</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein X is N, NH, or C;
- wherein Y is O or NH<sub>2</sub>;
- wherein A, B, and E are independently C, N, O, S, P, or Se;
- wherein n is 0 or 1; and
- wherein M is H<sub>2</sub>O or any metal.

51. The compound according to claim 50, wherein X is N and Y is NH<sub>2</sub>.

52. The compound according to claim 50, wherein X is C and Y is O.
53. The compound according to claim 50, wherein R is hydrogen, C1-C6 alkyl, C2-C6 alkynyl, C2-C6 alkenyl, benzyl substituted at from 0 to 3 positions in a chemically reasonable manner with F, Cl, Br, I, C1-C18 alkyl, Silyl, OH, OR', SH, SR', SOR', SO<sub>2</sub>R', SO<sub>3</sub>, or NR'<sub>2</sub>, or an amino acid and further wherein R' is H, OH, or alkyl;
54. The compound according to claim 50, wherein n = 0.
55. The compound according to claim 50, wherein n = 1 and further wherein M is a Group IA metal or a Group IIA metal
56. The compound according to claim 50, wherein M is Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, or Ca<sup>2+</sup>.
57. The compound according to claim 53, wherein R' is H, OH, C1-C6 alkyl, or C2-C6 alkynyl.

58. A compound of the formula VII:



- wherein A is NH<sub>2</sub>, OH, or O;

- wherein R is H, O, NR'<sub>2</sub>, S, CR'<sub>2</sub>, or halide;
- wherein R' is hydrogen or alkyl;
- wherein R<sub>3</sub> is either triphosphate,  $\alpha$ -thiotriphosphate, or a salt thereof;
- wherein L is alkyl;
- wherein X is CR or N and Y is O, S, or NH; and
- wherein the dye is any reporter group.

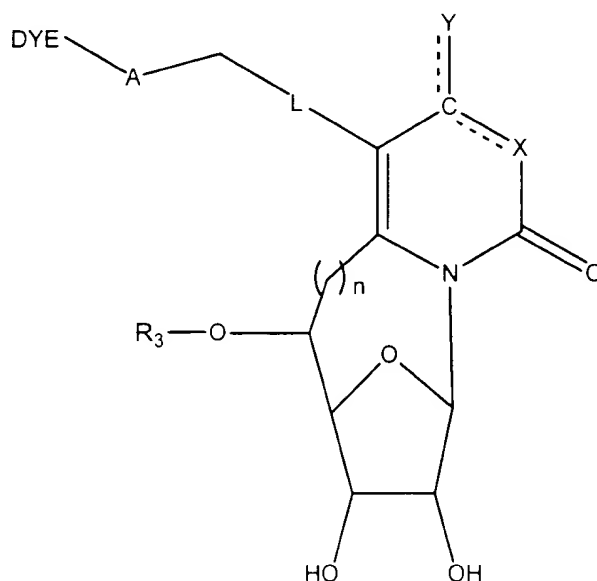
59. The compound according to claim 58, wherein R' is C1-C20 alkyl, C2-C20 alkenyl, or C2-C20 alkynyl, or aryl substituted at from 0 to 3 positions in a chemically reasonable manner with F, Cl, Br, I, C1-C18 alkyl, silyl, OH, OR', SH, SR', SOR', SO<sub>2</sub>R', SO<sub>3</sub>, or NR'<sub>2</sub>.

60. The compound according to claim 58, wherein R is fluorine, bromine, iodine, or chlorine.

61. The compound according to claim 58, wherein R is fluorine or chlorine.

62. The compound according to claim 58, wherein R' is hydrogen or C1-C7 alkyl.

63. A compound of the formula VIII:



Formula VIII

- wherein X is N, NH, or C;
- wherein Y is O or NH<sub>2</sub>;
- wherein R<sub>3</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein A is O, S, or NH;
- wherein L is alkyl or aryl substituted at from 0 to 3 positions in a chemically reasonable manner with F, Cl, Br, I, C1-C18 alkyl, Silyl, OH, OR', SH, SR', SOR', SO<sub>2</sub>R', SO<sub>3</sub>, or NR'<sub>2</sub>;
- wherein R' is hydrogen or alkyl;
- wherein n is 1 to 10; and
- wherein the dye is any reporter group, preferably a rhodamine-type dye, a fluorescein-type dye, an energy transfer dye, or a cyanine-type dye.

64. The compound according to claim 63, wherein L is C1-C20 alkyl, C2-C20 alkenyl, C2-C20 alkynyl, or benzyl substituted at from 0 to 3 positions in a chemically reasonable

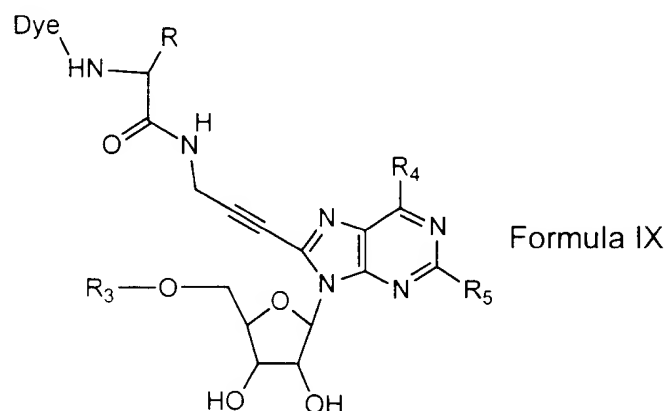
manner with F, Cl, Br, I, C1-C18 alkyl, Silyl, OH, OR', SH, SR', SOR', SO<sub>2</sub>R', SO<sub>3</sub>, or NR'<sub>2</sub>.

65. The compound according to claim 63, wherein R' is hydrogen or C1-C7 alkyl.

66. The compound according to claim 63, wherein X is N and Y is NH<sub>2</sub>.

67. The compound according to claim 63, wherein X is C and Y is O.

68. A compound of the formula IX:

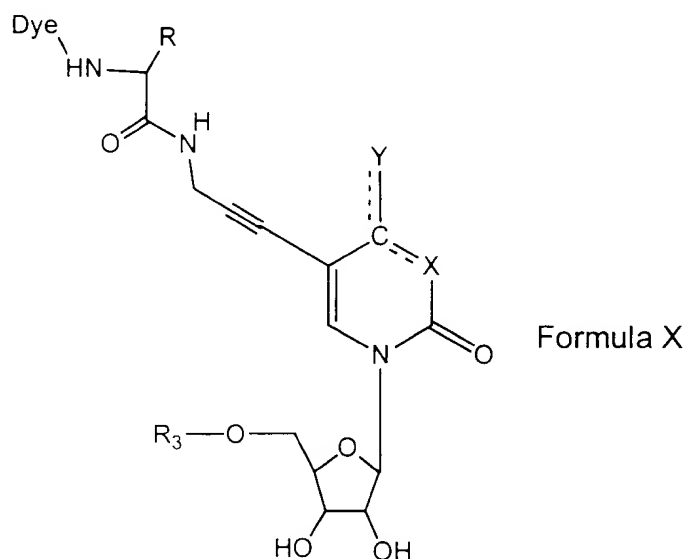


- wherein R<sub>4</sub> is NH<sub>2</sub>, OH, or O and R<sub>5</sub> is NH<sub>2</sub>, OH, or H, provided that if A is NH<sub>2</sub>, B is H and if A is O, B is NH<sub>2</sub>;

- wherein R<sub>3</sub> is either triphosphate, α-thiotriphosphate, or a salt thereof;
- wherein the dye is any reporter group; and
- wherein R is a side chain for mobility tuning.

69. The compound according to claim 68, wherein R is a hydrophilic side chain.

- 



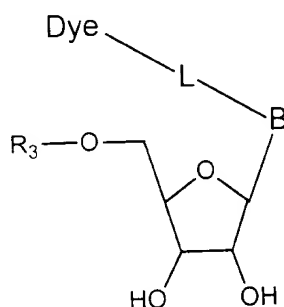
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75. The compound according to claim 73, wherein R is a hydrophobic side chain.

76. The compound according to claim 73, wherein X is N and Y is NH<sub>2</sub>.

77. The compound according to claim 73, wherein X is C and Y is O.

78. A compound having the formula:



- wherein B is a nucleobase selected from uracil, cytosine, adenine, 7-deazaadenine, guanine, and 7-deazaguanine;

- wherein R<sub>3</sub> is triphosphate or a salt thereof;

- wherein L is a linker selected from propargyl-ethyl-oxide-amino and propargylamino wherein the linker is attached to the 8-C of a adenine, 7-deazaadenine, guanine, or 7-deazaguanine nucleobase, the 7-C or 8-C of a 7-deazaadenine or 7-deazaguanine nucleobase, or the C-5 of a uracil or cytosine nucleobase; and

- wherein Dye is selected from a rhodamine dye and a fluorescein dye.

79. The compound according to claim 78, wherein B is uracil; L is propargyl-ethyl-oxide-amino; and Dye is TAMRA.



80. The compound according to claim 78, wherein B is uracil; L is propargylamino; and Dye is TAMRA.

81. The compound according to claim 78, wherein B is uracil; L is propargyl-ethyl-oxide-amino; and Dye is dTAMRA.

82. The compound according to claim 78, wherein B is uracil; L is propargylamino; and Dye is dTAMRA.

83. The compound according to claim 78, wherein B is cytosine; L is propargyl-ethyl-oxide-amino; and Dye is ROX.

84. The compound according to claim 78, wherein B is cytosine; L is propargylamino; and Dye is ROX.

85. The compound according to claim 78, wherein B is cytosine; L is propargyl-ethyl-oxide-amino; and Dye is dROX.

86. The compound according to claim 78, wherein B is cytosine; L is propargylamino; and Dye is dROX.

87. The compound according to claim 78, wherein B is 7-deazaadenine; L is propargyl-ethyl-oxide-amino; and Dye is R6G.

88. The compound according to claim 78, wherein B is 7-deazaadenine; L is propargylamino; and Dye is R6G.

89. The compound according to claim 78, wherein B is 7-deazaadenine; L is propargyl-ethyl-oxide-amino; and Dye is dR6G.

90. The compound according to claim 78, wherein B is 7-deazaadenine; L is propargylamino; and Dye is dR6G.

91. The compound according to claim 78, wherein B is 7-deazaguanine; L is propargyl-ethyl-oxide-amino; and Dye is R110.

92. The compound according to claim 78, wherein B is 7-deazaguanine; L is propargylamino; and Dye is R110.

93. The compound according to claim 78, wherein B is 7-deazaguanine; L is propargyl-ethyl-oxide-amino; and Dye is dR110.

94. The compound according to claim 78, wherein B is 7-deazaguanine; L is propargylamino; and Dye is dR110.

95. The compound according to claim 78, wherein Dye is an energy transfer dye pair comprised of a fluorescein dye and a rhodamine dye wherein the fluorescein dye is

attached to the nucleobase through a propargyl-ethyl-oxide-amino or propargylamino linker, and the fluorescein dye is attached to the rhodamine dye by a second linker.

96. The compound according to claim 95, wherein the fluorescein dye is 4-aminomethyl FAM and the rhodamine dye is a 4,7-dichlororhodamine dye.

97. The compound according to claim 96, wherein the 4,7-dichlororhodamine dye is dTAMRA.

98. The compound according to claim 96, wherein the 4,7-dichlororhodamine dye is dROX.

99. The compound according to claim 96, wherein the 4,7-dichlororhodamine dye is dR6G.

100. The compound according to claim 96, wherein the 4,7-dichlororhodamine dye is dR110.

Sub 21. A method for determining the sequence of a DNA template, comprising

- (i) annealing at least one oligonucleotide primer to a template;
- (ii) incubating said at least one oligonucleotide primer with a DNA polymerase that can incorporate both dNTPs and rNTPs in a reaction comprising a mixture of

unlabeled dNTPs and at least one dye-labeled ribonucleotide of the invention so that primer extension products are formed;

(iii) treating the primer extension products with a means for hydrolyzing the extension products at each occurrence of a ribonucleotide;

(iv) separating the resulting fragments that contain said at least one primer from other fragments,

(v) resolving the primer-containing extension products by size; and

(vi) detecting the fragments.

102. The method according to claim 101, wherein the dye-labeled ribonucleotides are rATP-PA-6R6G, rCTP-PA-Rox, rUTP-PA-Tamra and rGTP-EO-R110.

103. The method according to claim 101, wherein one primer is biotinylated.

104. The method according to claim 101, wherein at least one primer is a hybridization based pull-out primer.

105. The method according to claim 101, wherein the DNA polymerase is a thermostable DNA polymerase.

106. The method according to claim 105, wherein the thermostable DNA polymerase is a modified thermostable DNA polymerase having increased efficiency for the incorporation of ribonucleotides.

107. The method according to claim 101, wherein the means for hydrolyzing the extension products at each occurrence of a ribonucleotide is alkali treatment, heat treatment, or a ribonuclease.

108. A method for detecting mutations in DNA, comprising

- annealing two oligonucleotide primers to a template;
- incubating the two oligonucleotide primers with a DNA polymerase that can incorporate both dNTPs and rNTPs in a reaction comprising a mixture of unlabeled dNTPs and at least one dye-labeled ribonucleotide of the invention so that primer extension products are formed;
- treating the primer extension products with a means for hydrolyzing the extension products at each occurrence of a ribonucleotide to produce fragments;
- resolving the fragments by size; and
- detecting the fragments.

109. The method according to claim 108, wherein the fragments that contain primers are separated from other fragments before the fragments that contain primers are resolved by size.

110. The method according to claim 108, wherein the mutation is a single nucleotide polymorphism.

111. The method according to claim 108, wherein the DNA is genomic DNA.

112. The method according to claim 108, wherein at least one primer is biotinylated.
113. The method according to claim 108, wherein at least one primer is a hybridization based primer.
114. The method according to claim 108, wherein one primer comprises a modified base preventing primer extension in the 5' direction.
115. A method for preparing polynucleotide fragments, comprising
- incubating a DNA template with a DNA polymerase, dATP, dGTP, dCTP, dTTP, at least two oligonucleotide primers complementary to the DNA template, and at least one dye-labeled ribonucleotide so that the primers are extended and said at least one dye-labeled ribonucleotide is incorporated in the primer extension products; and
  - hydrolyzing 3'-5' phosphodiester linkages between adjacent ribo- and deoxyribonucleotides.
116. The method according to claim 115, wherein the 3'-5' phosphodiester linkages between adjacent ribo- and deoxyribonucleotides are hydrolyzed by alkali, heat, or a ribonuclease.
117. A method for preparing dye-labeled RNA complementary to a sequence of interest comprising:

- preparing a mixture of a template, an RNA polymerase, rATP, rGTP, rCTP, rUTP, and at least one dye-labeled ribonucleotide, wherein said sequence of interest is operably linked to a site for the initiation of RNA synthesis by the RNA polymerase; and
- incubating the mixture so that the RNA polymerase catalyzes the synthesis of RNA.

118. A method for detecting 5-methylcytosine in a DNA template comprising:

- treating the DNA template with a bisulfite salt under conditions whereby 5-methylcytosine remains non-reactive;
- incubating the DNA template with a DNA polymerase, dATP, dGTP, dCTP, dTTP, at least two oligonucleotide primers complementary to the DNA template, and a dye-labeled rCTP compound so that the primers are extended and the dye-labeled rCTP compound is incorporated in the primer extension products;
- hydrolyzing 3'-5' phosphodiester linkages between adjacent ribo- and deoxyribonucleotides to produce fragments;
- resolving the fragments by size; and
- detecting the fragments.

119. The method according to claim 118, wherein the template DNA is genomic DNA.

120. The method according to claim 118, wherein the fragments that contain primers are separated from other fragments before the fragments that contain primers are resolved by size.

121. The method according to claim 118, wherein at least one primer is biotinylated.

122. ~~The method~~ according to claim 118, wherein at least one primer is a hybridization based primer.

123. The method according to claim 118, wherein one primer comprises a modified base preventing primer extension in the 5' direction.